

## **Dual-Responsive Molecular Probe for Tumor Targeted Imaging and Photodynamic Therapy**

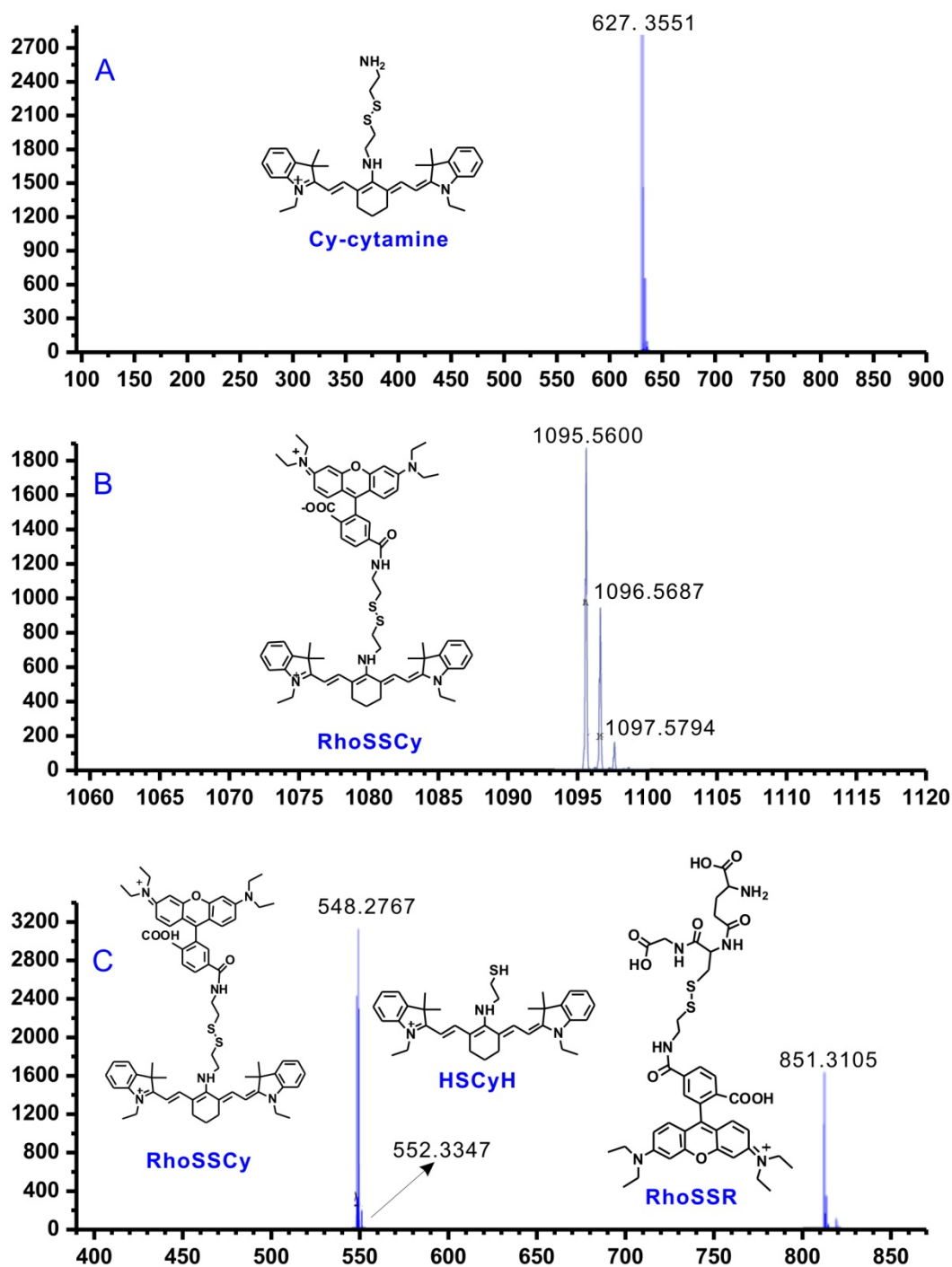
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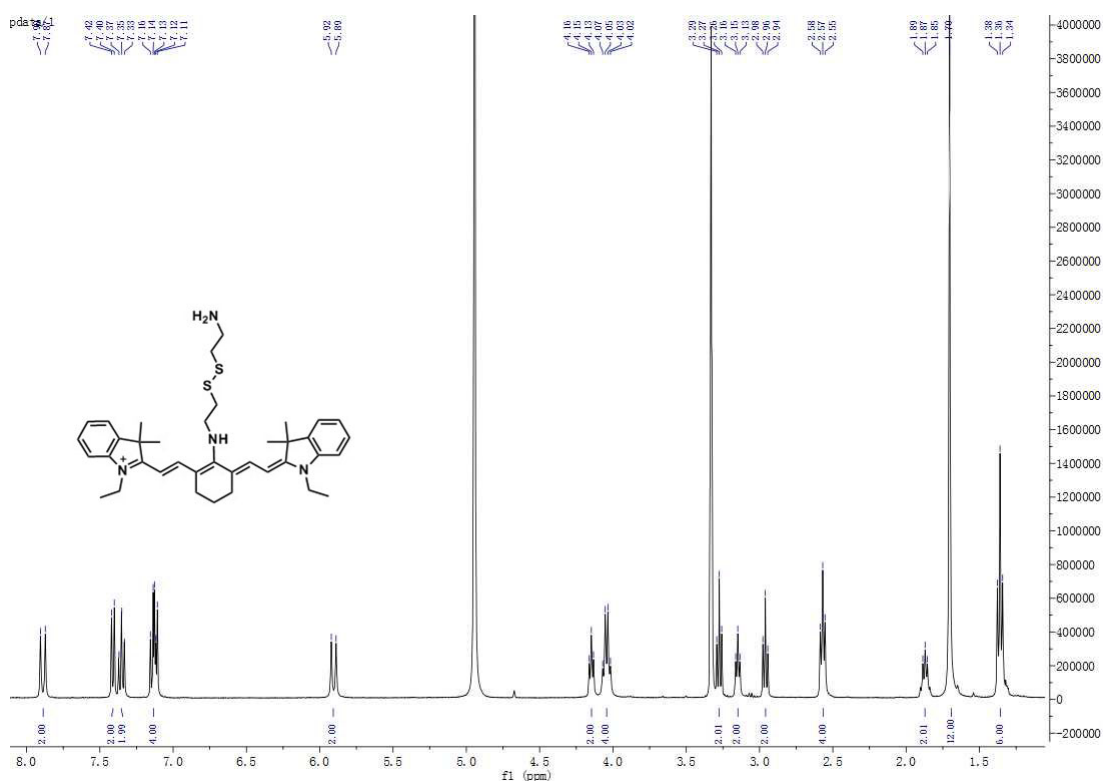
# Equal contribution

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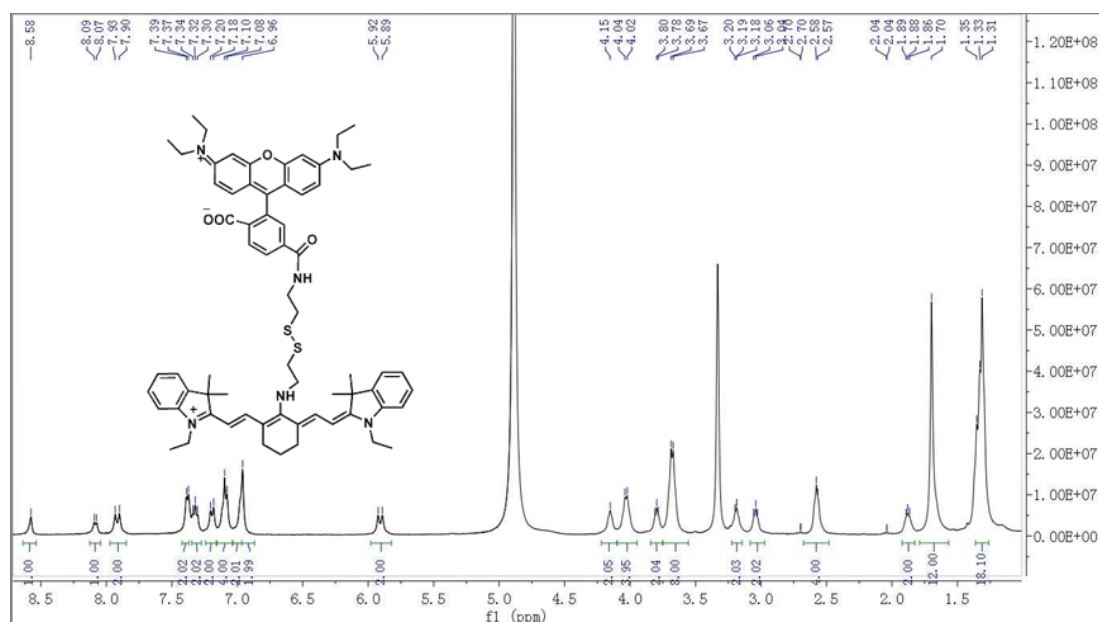
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**Figure S1.** Mass spectrum of compounds. (A) Cy-cytamine. HRMS (EI)  $m/z$   $C_{38}H_{51}N_4S_2^+$  ( $M^+$ ): 577.2666. Found 577.2654. (B) RhoSSCy. HRMS (EI)  $m/z$   $C_{67}H_{79}N_6O_4S_2^+$  ( $M^+$ ): 1095.5604. Found 1095.5600. (C) The mass spectra of RhoSSCy and the new molecules RhoSSR and HSCy. RhoSSCy HRMS (EI)  $m/z$   $C_{67}H_{80}N_6O_4S_2^{2+}$  ( $M^{2+}$ ): 548.2850. Found 548.2767. RhoSSR HRMS (EI)  $m/z$   $C_{41}H_{51}N_6O_{10}S_2^+$  ( $M^+$ ): 851.3103. Found 851.3105. HSCy HRMS (EI)  $m/z$   $C_{36}H_{46}N_3S^+$  ( $M^+$ ): 552.3407. Found 552.3347.

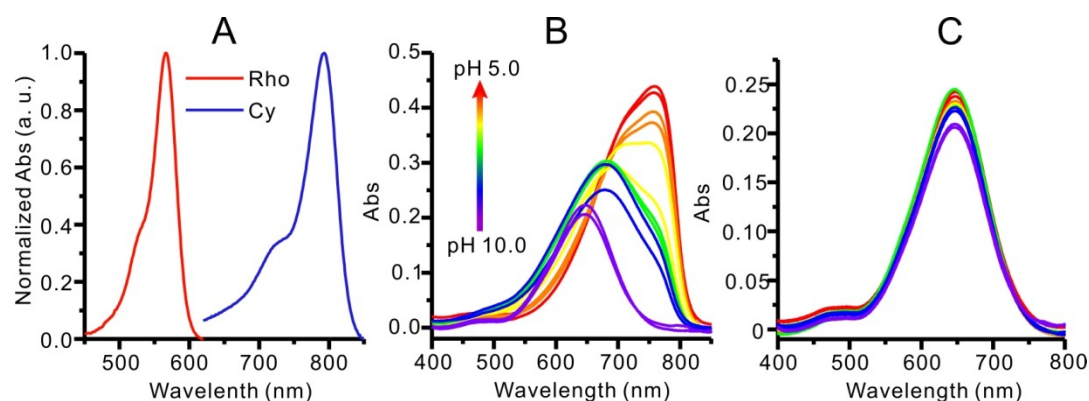


**Figure S2.** Cyss was confirmed by  $^1\text{H}$  NMR. (400 MHz,  $\text{CD}_3\text{OD}$ ),  $\delta$  7.90 (d,  $J=12.98$  Hz, 2H),  $\delta$  7.41 (d,  $J=5.50$  Hz, 2H),  $\delta$  7.35 (t,  $J=14.35$  Hz, 2H),  $\delta$  7.15 (d,  $J=9.16$  Hz, 2H),  $\delta$  7.12 (t,  $J=14.12$  Hz, 2H),  $\delta$  5.91 (d,  $J=12.59$  Hz, 2H),  $\delta$  4.15 (t,  $J=10.15$  Hz, 2H),  $\delta$  4.03 (q,  $J=10.46$  Hz, 4H),  $\delta$  3.27 (t,  $J=10.69$  Hz, 2H),  $\delta$  3.15 (t,  $J=11.85$  Hz, 2H),  $\delta$  2.96 (t,  $J=10.81$  Hz, 2H),  $\delta$  2.57 (t,  $J=10.81$  Hz, 4H),  $\delta$  1.87 (t,  $J=10.46$  Hz, 2H),  $\delta$  1.70 (s, 12H),  $\delta$  1.36 (t,  $J=7.05$  Hz, 6H)



**Figure S3.** RhoSSCy was confirmed by  $^1\text{H}$  NMR. (400 MHz,  $\text{CD}_3\text{OD}$ ),  $\delta$  8.58 (s, 1H),  $\delta$  8.08 (d,  $J=6.87$  Hz, 1H),  $\delta$  7.92 (d,  $J=12.98$  Hz, 2H),  $\delta$  7.38 (d,  $J=5.50$  Hz, 2H),  $\delta$

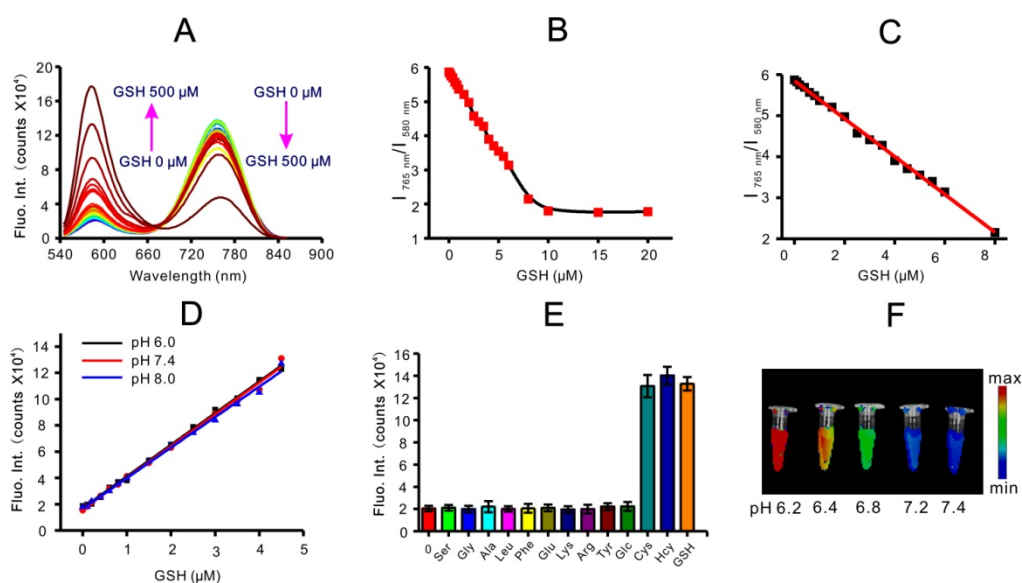
7.32 (t, J=14.35 Hz,2H),  $\delta$  7.19 (d, J=9.16 Hz,2H), $\delta$  7.10 (t, J =14.12 Hz, 4H),  $\delta$  6.98 (s, 2H),  $\delta$  6.96 (s, 2H),  $\delta$  5.91 (d, J = 12.59 Hz, 2H),  $\delta$  4.15 (t, J = 10.15 Hz, 2H),  $\delta$  4.03 (q, J = 10.46 Hz, 4H),  $\delta$  3.80 (t, J = 11.04Hz, 2H),  $\delta$  3.68 (d, J = 6.47 Hz, 8H),  $\delta$  3.19 (t, J = 10.69 Hz,2H),  $\delta$  3.04 (t, J = 11.85 Hz, 2H),  $\delta$  2.58 (t, J = 10.81 Hz, 4H),  $\delta$  1.88 (t, J = 10.46 Hz, 2H),  $\delta$  1.70 (s, 12H),  $\delta$  1.33 (t, J = 7.05 Hz, 18H)



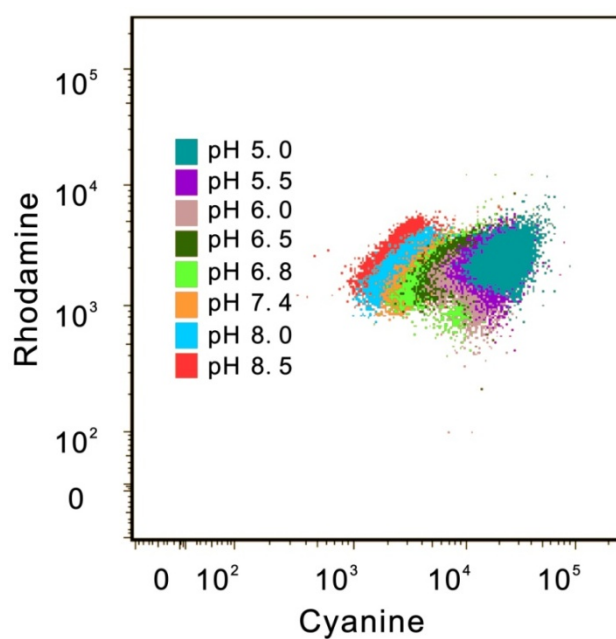
**Figure S4.** (A) UV-Vis absorption spectra of Rho and Cy. (B) UV-Vis absorption spectra of RhoSSCy at different pH (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0). (C) UV-Vis absorption spectra of RhoSSCy with different concentration of GSH (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 and 50  $\mu$ M).

**Table 1.** The fluorescence quantum yield of Rho group and a Cy group under different conditions

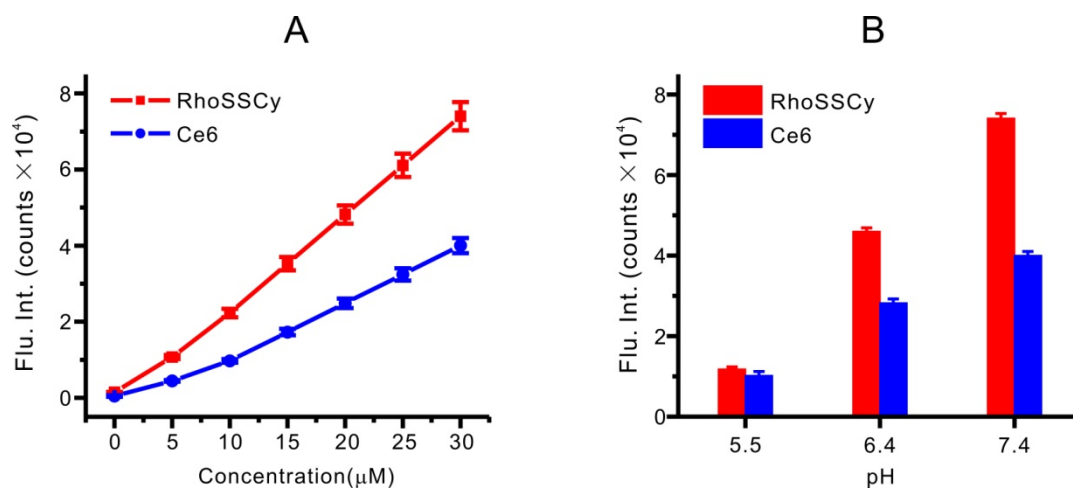
condition	structure	fluorescence quantum yield
Without GSH, PH 10.0	Rho group	0.01
	Cy group	0.007
Without GSH, PH 5.0	Rho group	0.01
	Cy group	0.14
With GSH, PH 10.0	RhoSSR	0.34
	HCyH	0.007
With GSH, PH 5.0	RhoSSR	0.34
	HCyH <sup>+</sup>	0.14



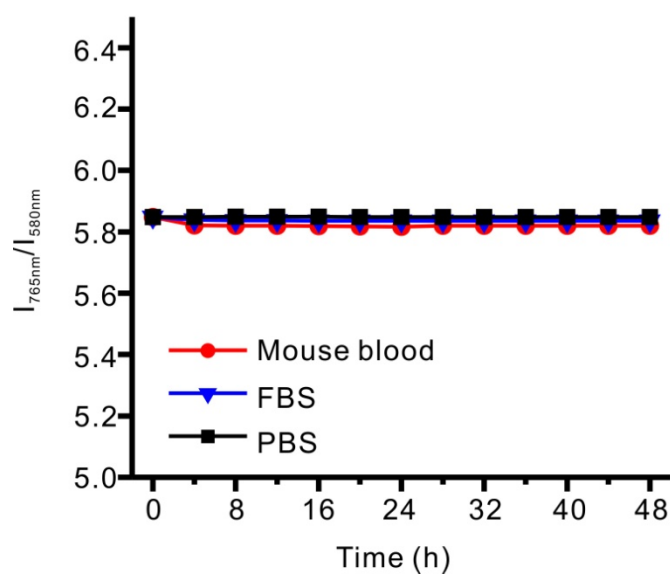
**Figure S5.** Fluorescence responses of RhoSSCy toward biothiols and pH. (A) Fluorescence spectra of 10  $\mu$ M RhoSSCy with different concentration of GSH upon excitation at 480 nm in 20 mM PBS (containing 1% DMF). (B) Fluorescence-based GSH titration curve ( $\lambda_{\text{ex}}$  at 480 nm). (C) The linear calibration plot of GSH titration. (D) Fluorescence intensity-based GSH titration curves with three different pH values ( $\lambda_{\text{ex}}$  at 480 nm and  $\lambda_{\text{em}}$  at 576 nm). (E) Fluorescence responses of RhoSSCy toward other analytes. (F) Fluorescence images of RhoSSCy in PBS buffer at different pH.



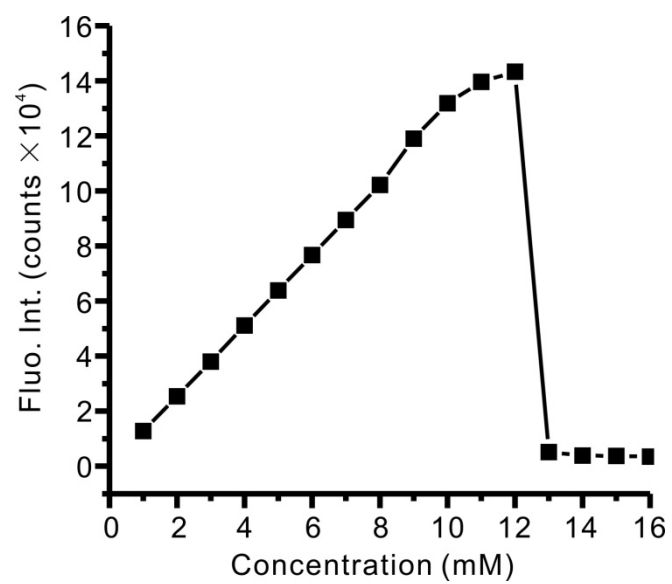
**Figure S6.** Flow cytometry analysis of pH response of RhoSSCy to MCF-7 cells.



**Figure S7.** ROS levels monitored through fluorescence intensity of DCF produced by RhoSSCy and Ce6. (A) The correlation between ROS levels and concentrations of RhoSSCy and Ce6. (B) The correlation between ROS levels and pH of RhoSSCy and Ce6 solution.



**Figure S8.** The ratio of fluorescent intensity at 765/580 nm in mouse blood, FBS and PBS within 48h.



**Figure S9.** Fluorescent intensity of RhoSSCy at different concentrations. ( $\lambda_{\text{ex}}$  at 640 nm,  $\lambda_{\text{em}}$  at 765 nm, slit width 0.1nm)